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STABILITY AND ANTIBACTERIAL ACTIVITY OF AG-TURI LEAF EXTRACT LIQUID SOAP FORMULA (*Sesbania grandiflora*)

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ABSTRACT

Ag is one of the metals proven to have antibacterial activity, so it is suitable for being converted into soap. On the other hand, the content of saponins and flavonoids can stabilize metals such as silver to support antibacterial effectiveness. This study aimed to make a soap formula of Ag-leaf turi to produce the stability of the soap formula used and antibacterial activity against candida A. bacteria that cause skin diseases. The method used is turi leaves, which are extracted using reflux. Ag nano-extract was prepared by reacting turi leaf extract with Ag. The Ag nano-extract is known to have a wavelength of 230nm. The formulation was prepared by varying the Ag nano-extract with 1, 2, and 3%. The physical tests carried out included pH with a pH stick, specific gravity with a pycnometer, viscosity with an Ostwald viscometer, and organoleptic by observing the color, smell, and shape of the soap that had been made. Antibacterial activity was used by the well method by looking at the clear zones produced. Meanwhile, the stability test was seen from the physical stability of the soap, which is adjusted to the formula that has been made. The results from these research that has been implemented, it can be reported that the soap from the Ag nano-extract based on physical properties such as organoleptic, viscosity test, specific gravity, pH and homogeneity has met the requirements.

Keywords: Ag-Turi Leaf, Soap Formula, Stability, Antibacterial.

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INTRODUCTION

The liquid soap formulation is the choice because it is more practical in use and has a more attractive appearance.[1] Liquid soap is formulated to be used as a cleaning agent when bathing and washing hands.[2] Liquid soap uses the active ingredient potassium hydroxide, which is mixed with oil.[3] Liquid soap can remove dirt due to the emulsion formed from soap with dirt. So that dirt in the form of fat can be lifted from the surface of the skin. Fat or oil that is carried along with dirt on the skin will, of course, cause dry skin and not be protected from bacteria or germs. Therefore, the soap formula is added with other active substances that function as skin protectors. The active substances in soap are able to protect the skin from germs such as bacteria or fungi. Besides that, it can also function as a beauty ingredient, such as retaining skin moisture by adding active plant ingredients.[4] This aloe vera-based liquid soap has the ability to inhibit the growth of several gram-positive bacteria, including *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, and Bacillus cereus*, as well as gram-negative bacteria, such as Salmonella typhimurium, Proteus mirabilis, Pseudomonas aeruginosa, and Escherichia coli.[5] His report provides education about making soap from betel leaves, which can clean the female area. The development of technology for making soap with active plant ingredients as an effort to increase the effectiveness of soap in fighting germs is increasing from year to year. One of the efforts to increase the effectiveness of soap is by using nanotechnology.[6] Nanoparticles are an attempt to produce materials up to nanometers in size.[7] Liquid soap with nanotechnology has been developed, such as nano liquid soap from virgin coconut oil with bengkoang tuber filtrate active substance.[8] Besides that, nano anti-acne was developed from the chitosan extract of mangosteen fruit.[9] Nano technology was also developed by making chitosan serum from Centella asiatica.[10] So that the formulation of liquid soap based on nanotechnology needs to be done, one of which is the active substance from turi leaf extract. Turi leaves contain active ingredients that can support the formulation of liquid soap based on nanotechnology. Turi leaves contain saponins and flavonoids.[11]

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This content is also supported by the ability of turi leaves to stabilize metals, such as silver, to support antibacterial effectiveness.[12] In a literature search, there are still few reports regarding the formulation of liquid soap with the active ingredient Ag-turi leaf extract. In addition, there is a shortage of soap made from turi, which has an unpleasant odor. So, research is needed to find a liquid soap formula that meets SNI standards. In addition, liquid soap using nanotechnology is expected to increase the penetration of antibacterial activity.

EXPERIMENTAL

Methods

The materials that have been used in this study include turi leaves, $AgNO_3$ p.a, distilled water, EtOH p.a, Whatman filter paper, activated charcoal, disc paper, NB, NA, and S. Epidermal bacteria suspension.

Preparation of Turi Leaf Samples

Turi leaves are washed using distilled water, cut into small pieces, and then dried in an oven. Once dry, the leaves are blended into powder and sieved using a 50-mesh sieve.

Sample Extraction

The dried sample was then mashed with a pollination rate of 40 mesh escape. The simplisia powder was then extracted using reflux with a sample and solvent ratio of 96% ethanol, which is 1:10 for two hours with a constant temperature of 80°C. During the synthesis process, the solution turned light brown. After that, the prepared extract was cooled to room temperature and then filtered using Whatman filter paper.

Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using a method involving the reduction of silver ions using plant extracts as bioreductants. A solution containing 0.5 mM or 1.5 mM $AgNO_3$ was mixed with 1 mL of turi leaf extract and stirred using a magnetic stirrer for one day until the color of the solution changed from yellow to brownish. The formation of silver nanoparticles was confirmed through this visual change, which was further characterized using UV-Vis spectroscopy to determine its maximum absorbance and wavelength.[13]

Characterization of Silver Nanoparticles UV-Vis Spectrophotometer

Silver nanoparticle characterization was carried out using a UV-Vis spectrophotometer to confirm their formation and assess their stability. UV-Vis spectroscopy was carried out in the wavelength range of 300–600 nm using a quartz cuvette with distilled water as a blank. The silver ion reduction process was confirmed through the obtained UV-Vis spectrum.[14] Colloidal silver nanoparticles are formed with plasmon resonance peaks ranging from 400 nm to 450 nm (Table- 1).

Table- 1: Liquid Soap Formulation

Material	Unit	Formula		
		1	2	3
Ag nano-extract	mL	1	2	3
Olive oil	mL	15	15	15
KOH	mL	8	8	8
CMC	Gram	1	1	1
SLS	Gram	15	15	15
stearic acid	Gram	7,5	7,5	7,5
AHA	Gram	0,5	0,5	0,5
Aquades	mL	100	100	100

Test the Physical Properties of Turi Leaf Extract Nanoparticle Soap

The physical properties test of solid soap includes: organoleptic test, pH test, stability test and foam height, water content test, total fatty acid test, and free alkali test.

Size Test with Particle Size Analyzer

Tests have been carried out with a particel size analyzer (Horiba Scientific SZ-100) with conditions of scattering angle 173, temperature of the holder 25 °C, dispersion medium viscosity 0,894 mPa.S, tranmission intensity before measurement 1206, distribution form standar, distribution form (disperity) with polydisperse, representasion of result with scattering light intensity, and count rate 11013 kCPS.

Antibacterial Activity Test

BHI media is a medium for breeding S. Epidermis bacteria. MHA media was used as a medium for the use of antibacterial activities by the well method. Then making a well with a diameter of 15 mm to be filled with each formula and then waited for 1x24 hours in the incubator. Next, calculate the clear zone as an antibacterial resistance zone following the area of the circle formed.

RESULTS AND DISCUSSION

Sample Preparation

Green turi leaves are used as samples, measuring 3-5 cm (Fig.- 1). These leaves are obtained in the morning to obtain high metabolite content. The turi leaf extract obtained was then tested for flavonoids by reacting it first with $FeCl_3$ to produce a purple color, which showed a positive result. These results were in line with Asmara (2017) research that turi leaves contain flavonoids. Besides that, turi leaves also contain polyphenols, steroids, saponins, and alkaloids.[15] Turi leaves were known to contain metabolites such as nitrogen, protein, and minerals such as calcium and phosphorus.[16]

Fig.- 1. Cross-section of Turi Leaves (*Sesbania grandiflora*)

The turi leaves used in this study were turi leaves originating from the Yomani area, Tegal Regency. The characteristics of turi leaves are a stem length of 25 cm and leaves measuring 4.5 cm.

Sample Treatment

The initial treatment of turi leaves obtained is to do a rough sorting to separate turi leaves which are still green and yellow or those which have a yellow color. Loss of green color in turi leaves is known that these leaves have started to age and lose metabolites such as chlorophyll .

Fig.- 2: Drying Process of Turi Leaves (*Sesbania Grandiflora*)

The dried turi leaves were marked by the fading of the green color. Then, the dried turi leaves were tested for water content and obtained an average water content of 9.8% by the weighing method by doing constant weighing.

Microscopic Test of Turi Leaves

Tests for the correctness of plants are used to provide objective values from the samples used.[17] Meanwhile, the microscopic test is intended to give special characteristics to the samples used.[18] Turi leaves have

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distinctive characteristics with the presence of cork, parenchyma, fiber bundles with oxalate crystals, and oxalate crystals as a typical metabolic product of the leaves. The results of the microscopic test of turi leaves are presented in the (Fig.- 3) below:

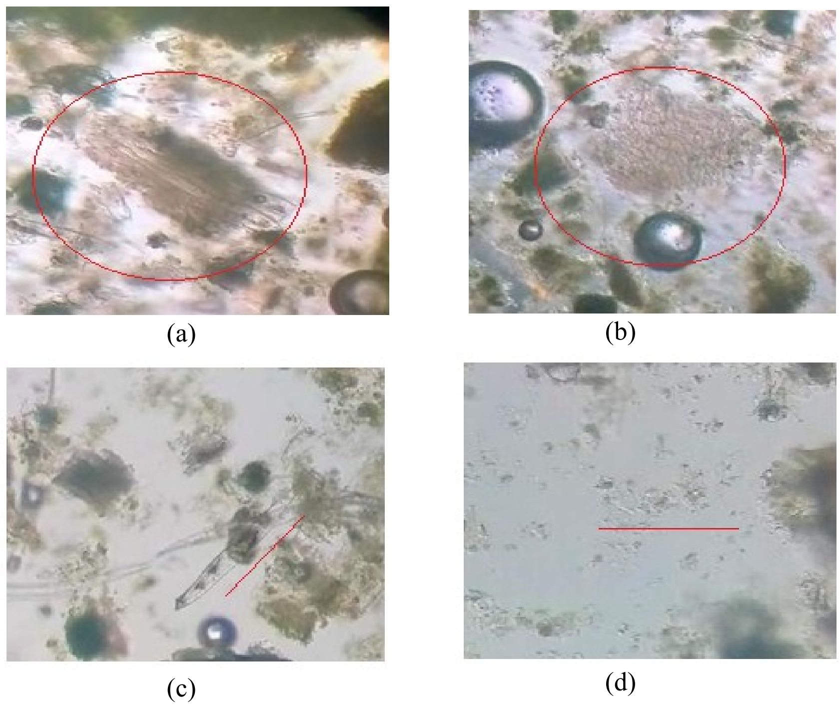

Fig.- 3: Microscopic Examination of Turi Leaves: (a) Cork, (b) Parenchyma, (c) Fiber Bundles with Oxalate Crystals, and (d) Oxalate Crystals

Corky tissue was characteristic of the turi plant. This network was formed from felogen, which serves to protect the underlying tissue, and this tissue is also used to store the oxygen content of the leaves.[19] Parenchyma is a tissue in the leaf that functions to absorb sunlight for the photosynthesis process.[20]

Sample Extraction

Ethanol solvents were used because of ethanol's ability to attract various methoglyces in various polarities. The presence of hydroxyl groups can attract other polar groups, such as flavonoids.[21] The extract that has been obtained was then filtered until liquid extract. Then, it was dried by using a rotavapor until a dry extract was obtained with a percentage of 1.18% (Fig.- 4).

Turi leaf extract (*Sesbania grandiflora*) (added 3-4 drops of $FeCl_3$ solution and positive for flavonoid content resulting in a brown color. This is in line with research that the flavonoid content can be reacted with a metal such as extracts and fractions from male papaya leaves, which is reacted with a mixture of magnesium and hydrogen chloride to produce a red-orange color. [21] The metal will experience reduction in the presence of flavonoids and will produce a distinctive color.

Preparation of Ag-Turi Leaf Extract

Bireductors from plants can be used in various ways, one of which is for soap formulations. Plant extracts are capable of reducing metals to nano-size. This reduction ability is certainly inseparable from the content of the plant itself, such as secondary metabolites. These metabolites can be obtained by extraction, which is the withdrawal of components with a suitable solvent.[22] Metals that can be used to be reduced by plant components include Ag. Ag is one of the metals that has been widely used for medical devices due to its antibacterial ability.

In addition, Ag is cheaper than gold (Au), so it has the potential to be used and can reduce production costs.[23] The extract that has been obtained is then used as a bioreductor to reduce Ag+ ions to Ag so that an Ag-extract with nano size will be formed. Various plant secondary metabolites have been used for plant

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bioreductors. Anthocyanins from *Clitoria ternatea*, curcumin from Curcuma, Gallic acid and quercetin from malva vertie blata leaves, phenolics from *petroselinum crispum* leaves, and flavonoids from *glyzyrrhzia glabra*. These metabolites constitute one class, namely, the phenolic class. Phenolic is a functional group that can release hydroxy ions that can reduce metals.[22] Flavonoids (Fig.- 5) have detachable hydroxy ions that have the ability to bind Ag ions to form a complex so that they experience reduction due to the donation of electrons from the hydroxy groups of flavonoids.[24]

Fig.- 4 Turi Leaf Extract (*Sesbania grandiflora*)

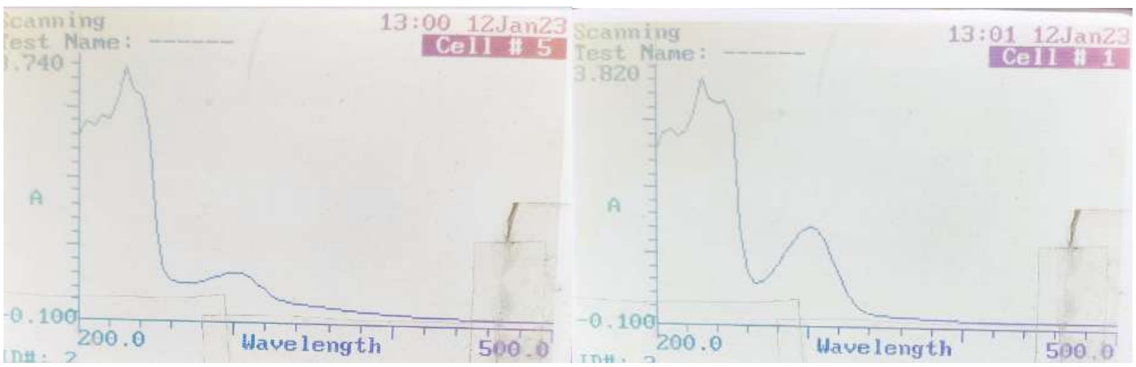

Fig.- 5: Maximum Wavelength Scanning of Ag Nano-Extract a) 0.5 µM Nano Ag Concentration b) 1.5 µM Ag Nano Concentration

Maximum wavelength scanning data from extract concentrations of 0,001 gram with 0,5 and 1,5 µM Ag solutions at 230 nm with absorbances of 3.565 nm and 3.641 nm, respectively. The particle sizes obtained were 96 and 102 nm, respectively, using the particle size analyzer method. This report was in line with Fabiani et al. (2019) that Ag-nano is in the range of 200-400 nm with crystalline from XRD results.[25] However, it is different in that the size of the crystals formed is smaller at 23 nm. This can occur if there is still Ag that has not been perfectly reduced, which causes the Ag formed to still be large. This happens if the flavonoids that react with Ag are less.[25-26]

Ag Nano-Extract Liquid Soap Formulation

The liquid soap made is a liquid soap based on caustic oil. The type of oil used is olive oil, and the caustic used is KOH. Turi leaf extract that has been synthesized forms Ag nano-extract, which is formulated into soap with a concentration of 1, 2, and 3 mL, respectively, with an Ag concentration of 0.5µM. The formulation of Ag nano-extract liquid soap (Table- 2).

The soap-making activity begins with weighing all the ingredients needed to make soap. Soap making begins with developing CMC by heating water first until the temperature reaches 70°C. The CMC is then mixed with the chili sauce, stirring continuously until the CMC is completely dissolved and there are no lumps. The next step is to add stearic acid and stir until evenly distributed and homogeneous. The next step is to add SLS, olive oil, and KOH successively with continuous stirring until evenly distributed. The final step is adding AHA and the active ingredient Ag nano-extract. After the mixture is homogeneous, add water according to the dosage of the soap formula and stir again until the soap has no lumpy components. Soap making was carried out up to three times as replication and continued with all soap formulas.

Table- 2: Formulation of Ag Nano-Extract Soap

Material	Unit	Formulas		
		1	2	3
Ag nano-extract	mL	1	2	3
Olive oil	mL	15	15	15
KOH	mL	8	8	8
CMC	gram	1	1	1
SLS	gram	15	15	15
Stearic acid	gram	7,5	7,5	7,5
AHA	gram	0,5	0,5	0,5
Aquadest	mL	100	100	100

Fig.- 6: Ag Nano-Extract Liquid Soap

Liquid soap using KOH base with olive oil was used in this study. Ag nano-extract functions as an active substance that can increase penetration power in antibacterial activity and dirt-cleaning processes.[26] Other substances, such as SLS, are used as foaming agents and act as surfactants.[27] In making soap, the caustic system and oil cannot combine, so the addition of stearate can reduce surface tension due to the formation of an emulsion before the soap is formed (Fig.- 6).[2]

Physical Test of Ag Nano-Extract Liquid Soap

The soap that had been made was then tested for its physical properties, including organoleptic, pH, specific gravity, viscosity, foaming power, and homogeneity. The physical test is intended to measure experimental results for compliance with standards. The first thing to do is the organoleptic test, which includes the shape, color, and smell of the soap. Homogeneity test to find out that all the soap-forming components have been thoroughly mixed (Table-3)

Table-3: Physical Test Results of Ag. Nano-Extract Soap

Test result	Formula 1	Formula 2	Formula 3
Test results Form Color Smell	 Liquid White No smell	 Liquid White No smell	 Liquid White No smell
pH	6	6	6
density	0,974±0,003	0,986±0,006	0,988±0,03
Foam power (mm)	150±2	155±1,5	160±2
Homogenitas	Homogeneous	Homogeneous	Homogeneous
Viscosity (cp)	25,3±2,1	26,8±1,4	28,4±3,0
Bacterial inhibition zone	4,5±1,0 mm	5 ± 0,5mm	6 ± 1,3mm

The antibacterial properties of Ag-turi leaf soap against S. Epidermis produce an inhibition of 4.5 to 6 mm (Fig.- 7). The inhibition of soap increases with the increase in the concentration of Ag-turi leaf extract. This is in line with research from Rusiana (2024), as turi has antibacterial activity against staphylococcus aureus Bacteria.[28]

CONCLUSION

From the results of the exploration that has been carried out, it can be concluded that the soap from turi leaf extract reacted with Ag has met the requirements to be formulated into soap with the potential to be made into a soap product that was suitable for production and further testing.

Fig.- 7: Antibacterial Activity of the Well Method

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest in this manuscript.

AUTHOR CONTRIBUTIONS

All authors contributed significantly to the preparation of this manuscript, participated in the review and editing process, and approved the final draft for publication. The research profile of each author can be verified through the ORCID ids listed below:

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